

A Structural Analysis of Directed Evolution Products

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Directed evolution provides a powerful tool to answer biochemical questions involving many aspects of protein stability and function. It allows the experimenter to rapidly and simultaneously screen sequence, structure, and function space for mutant proteins with desired characteristics. Gene shuffling and ensures that all or most silent or deleterious mutations are removed, resulting in mutant proteins where the role of each mutation is, presumably, significant and instructive as a biochemical solution as a defined environment. Rather than relying on sequence or structural alignments of proteins from different species, in which the bulk of the mutations may have arisen from genetic drift and be functionless, directed evolution allows investigation of the minimal sequence change necessary to produce the desired phenotypic change. Additionally, mutations favored in response to multiple different selection pressures can be investigated. This permits a structural view of evolutionary divergence in a setting where the selection pressure, startpoint, pathway, and endpoint are all well defined.

In work recently completed at the ALS, we determined the three dimensional structures of three para-Nitrobenzyl (PNB) esterases that were evolved to possess unique activities and biophysical characteristics. The structures were solved with phases determined from a 3 wavelength mercury MAD experiment performed on crystals of the organophile at the ALS. The MAD data were collected in less than 4 hours. In addition to allowing the experimenter to tailor the activity of an enzyme, directed evolution products present interesting targets for structural study. In this work, the structures of the products of two orthogonal screens, one for thermostability and one for organic activity, were determined. The organophile (56c8) is 100 times more active toward the p-nitrobenzyl ester of the antibiotic loracarbef (pNB-LCN) in 25% dimethylformamide than the wild type enzyme, and the thermophile (8g8) is 17 °C more stable than wild type. Structural analysis reveals these characteristics to be the result of groups of interacting mutations that rigidify surface loops and reshape the active site of the serine hydrolase.

pNB esterase is a 489 amino acid α/β protein of composed of a central 13 stranded Beta sheet surrounded by 15 alpha helices. The evolved proteins are very similar to the wild type with root mean squared deviations of 0.67 Å and 0.44 Å for the organophile and thermophile respectively. The most striking changes occur in four surface loops (66-74, 265-275, 315-324 and 414-420) that form the entrance to the active site as well as forming a face of the active site cavity.

Shown at right is a ribbon diagram of the recently solved pNB esterase. The structure is viewed looking into the active site cavity. The



structure is colored by RMSD showing regions that reorganize substantially in red and regions that are unaffected by the selection pressure in blue. Two loops that are not structured in the wild type but well ordered in 56c8 and 8g8 are shown as dashed lines. The side chains of the catalytic triad are shown in light blue.

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